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Gary V. Doern

*Et al.*

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## Multicenter Laboratory Evaluation of the bioMerieux Vitek Antimicrobial Susceptibility Testing System with 11 Antimicrobial Agents versus Members of the Family *Enterobacteriaceae* and *Pseudomonas aeruginosa*

GARY V. DOERN,<sup>1\*</sup> ANGELA B. BRUEGGEMANN,<sup>1</sup> ROCCO PERLA,<sup>1</sup> JUDY DALY,<sup>2</sup>  
DEMETRIOS HALKIAS,<sup>3</sup> RONALD N. JONES,<sup>4</sup> AND MICHAEL A. SAUBOLLE<sup>5</sup>

University of Massachusetts Medical Center, Worcester, Massachusetts 01655<sup>1</sup>; Primary Children's Medical Center, Salt Lake City, Utah 84113<sup>2</sup>; Veterans Administration Medical Center, Tampa, Florida 33612<sup>3</sup>; University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242<sup>4</sup>; and Good Samaritan Hospital, Phoenix, Arizona 85062<sup>5</sup>

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A four-center study in which a total of 1,082 recent clinical isolates of members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* were examined versus 11 antimicrobial agents with the bioMerieux Vitek susceptibility test system (Hazelwood, Mo.) and the GNS-F6 card was conducted. In addition, a challenge set consisting of the same 200 organisms was examined in each of the four participating laboratories. Results obtained with the Vitek system were compared to MICs determined by a standardized broth microdilution method. For purposes of comparison, susceptibility categories (susceptible, intermediate, or resistant) were assigned on the basis of the results of both methods. The result of the broth microdilution test was considered definitive. The total category error rate with the Vitek system and the recent clinical isolates (11,902 organism-antimicrobial comparisons) was 4.5%, i.e., 1.7% very major errors, 0.9% major errors, and 1.9% minor errors. The total category error rate calculated from tests performed with the challenge set (i.e., 8,800 organism-antimicrobial comparisons) was 5.9%, i.e., 2.2% very major errors, 1.1% major errors, and 2.6% minor errors. Very major error rates higher than the totals were noted with *Enterobacter cloacae* versus ampicillin-sulbactam, aztreonam, ticarcillin, and ticarcillin-clavulanate and with *P. aeruginosa* versus mezlocillin, ticarcillin, and ticarcillin-clavulanate. Major error rates higher than the averages were observed with *Proteus mirabilis* versus imipenem and with *Klebsiella pneumoniae* versus ofloxacin. Excellent overall interlaboratory reproducibility was observed with the Vitek system. The importance of inoculum size as a primary determinant in the accuracy of susceptibility test results with the Vitek system was clearly demonstrated in this study. Specifically, when an inoculum density fourfold higher than that recommended by the manufacturer was used, high rates of false resistance results were obtained with cell wall-active antimicrobial agents versus both the *Enterobacteriaceae* and *P. aeruginosa*.

The Vitek system (bioMerieux Vitek, Hazelwood, Mo.) is an automated method for performing same-day identification and antimicrobial susceptibility tests on nonfastidious bacteria. It was first introduced in 1979 and has received broad application in clinical microbiology laboratories worldwide. Numerous published reports have described the antimicrobial susceptibility testing capability of the Vitek system. Many of these studies have addressed susceptibility testing of *Enterococcus* species (2, 8, 16, 22–25, 27, 29) or staphylococci (3, 4, 7, 8, 14, 26, 30, 31). Several published investigations have described acceptable levels of accuracy when the Vitek system was used to determine the antimicrobial susceptibility patterns of members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* (6, 9–11, 13, 19–21, 28, 32). A single exception might be detection of extended-spectrum  $\beta$ -lactamase-producing *Klebsiella* and *Escherichia* species with current test materials (12). Recently, two reports that describe high rates of false resistance with imipenem and aztreonam when selected *Enterobacteriaceae* and *Pseudomonas* spp. were tested with the currently available Vitek system have appeared (1, 15). Because of these two

reports, a prospective, controlled multicenter study was conducted in an effort to assess the accuracy of the contemporary Vitek system as a means for determining the antimicrobial susceptibility of clinical isolates of facultative gram-negative bacilli.

### MATERIALS AND METHODS

**Organisms tested.** Between 231 and 320 unique patient isolates of *Enterobacteriaceae* and *P. aeruginosa* were examined in each of four medical centers: Good Samaritan Hospital, Phoenix, Ariz.; Veterans Administration Medical Center, Tampa, Fla.; University of Iowa Hospitals and Clinics, Iowa City, Iowa; and Primary Children's Medical Center, Salt Lake City, Utah (Table 1). In addition, a collection of 200 common stock strains was examined in each center (Table 1).

**Study design.** Antimicrobial susceptibility tests were performed in each of four laboratories using the GNS-F6 card and the bioMerieux Vitek instrument according to the manufacturer's instructions. Inocula of clinical isolates were prepared directly from primary plates. The 200 isolates in the common challenge set were tested after two subcultures. The following 11 antimicrobial agents were used: ampicillin, ampicillin-sulbactam, aztreonam, ciprofloxacin, imipenem, mezlocillin, ofloxacin, piperacillin, ticarcillin, ticarcillin-clavulanate, and trimethoprim-sulfamethoxazole (TMP-SMX). After testing, all of the clinical isolates were shipped on tryptic soy agar slants to the University of Massachusetts Medical Center (UMMC), where stock cultures were prepared and frozen at  $-70^{\circ}\text{C}$ . Stock cultures of the challenge set were also maintained at UMMC at  $-70^{\circ}\text{C}$ .

At UMMC, both clinical isolates and strains from the challenge set were subsequently reconstituted from frozen stocks and subcultured twice on sheep blood agar plates incubated at  $35^{\circ}\text{C}$  for 20 to 24 h, and MICs were determined with the same 11 antimicrobial agents tested with the GNS-F6 card (see above).

\* Corresponding author. Mailing address: Clinical Microbiology, Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242. Phone: (319) 356-8616. Fax: (319) 356-4916.

TABLE 1. Clinical isolates and common stock strains examined by both Vitek GNS-F6 and broth microdilution methods

Organism	No. of strains				
	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Common stock
<i>Escherichia coli</i>	41	40	40	42	30
<i>Proteus mirabilis</i>	36	40	40	11	16
<i>Klebsiella pneumoniae</i>	36	40	40	38	20
<i>Enterobacter cloacae</i>	33	50	40	21	20
<i>Pseudomonas aeruginosa</i>	36	40	39	43	32
<i>Shigella sonnei</i>	2			26	1
<i>Shigella boydii</i>	1				
<i>Shigella dysenteriae</i>	1				
<i>Shigella flexneri</i>	1			5	
<i>Salmonella</i> spp.	1		1	15	
<i>Citrobacter amalonaticus</i>				1	
<i>Citrobacter freundii</i>	6	19	11	13	
<i>Citrobacter koseri</i>	6	7	1	1	
<i>Klebsiella oxytoca</i>	1	2	20	10	6
<i>Klebsiella ozaenae</i>					1
<i>Enterobacter aerogenes</i>	10	24	15	2	18
<i>Enterobacter agglomerans</i>	1	1		1	2
<i>Enterobacter amnigenus</i>	1				
<i>Enterobacter sakazakii</i>				1	
<i>Hafnia alvei</i>		1			1
<i>Serratia liquefaciens</i>		1			3
<i>Serratia marcescens</i>	5	20	18		15
<i>Serratia odorifera</i>					1
<i>Proteus vulgaris</i>	3	3	6		8
<i>Providencia rettgeri</i>	1	1	6		9
<i>Providencia rustigianni</i>					2
<i>Providencia stuartii</i>		25	7		3
<i>Pseudomonas alcalifaciens</i>	1				
<i>Morganella morganii</i>	5	6	12	2	2
<i>Lecelercia adecarboxylata</i>	1				
<i>Acinetobacter calcoaceticus</i>	2		3		7
<i>Acinetobacter lwoffii</i>					1
<i>Aeromonas hydrophila</i>					2
Total	231	320	299	232	200

MICs were determined by a microdilution method in cation-adjusted Mueller-Hinton broth (100- $\mu$ l final volume per well) according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (18). The final inoculum concentration was ca.  $5 \times 10^5$  CFU/ml. Antimicrobial powders were obtained from their respective manufacturers. The following control organisms were employed: *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853.

**Categorization of errors.** The susceptibility category (susceptible [S], intermediate [I], or resistant [R]) assigned to each organism-antimicrobial combination by the Vitek system was taken as the Vitek result. The MIC results obtained with the broth microdilution method were converted into susceptibility categories (S, I, or R) according to the interpretive criteria of the NCCLS (18) and considered to be definitive. Very major errors were defined as an S result with Vitek GNS-F6 and an R result according to the MIC, the opposite pattern was considered a major error, and any other category discrepancy was defined as a minor error.

## RESULTS

The results of testing of clinical isolates in the four participating laboratories are depicted in Table 2. Several observations could be made from these data. In general, there was excellent concordance among the four laboratories with respect to error rates for individual antimicrobial agents. Furthermore, in no case either for data from individual laboratories or for the data analyzed collectively was the aggregate error rate (i.e., very major plus major plus minor errors) for a specific agent found to exceed 10%. Three agents were found to yield total aggregate error rates of <3.0%: ciprofloxacin (1.4%), ofloxacin (2.6%), and TMP-SMX (2.2%). Total aggregate error rates of 3 to 7% were observed with seven drugs:

ampicillin (3.2%), aztreonam (4.6%), imipenem (3.4%), mezlocillin (6.2%), piperacillin (6.8%), ticarcillin (4.7%), and ticarcillin-clavulanate (6.5%). The final agent, ampicillin-sulbactam, yielded a total aggregate error rate of 8.4%. The overall aggregate error rate calculated from the total of 1,082 clinical isolates tested versus 11 antimicrobial agents with the GNS-F6 card was 4.5% (1.7% very major, 0.9% major, and 1.9% minor errors).

The distributions of errors in each of the three error categories were roughly equivalent for most antimicrobial agents (Table 2). Essentially the same observations could be made from the results of testing of a set of 200 common challenge strains in the four laboratories (data not shown).

As seen in Table 1, five species were tested in numbers large enough to permit analysis individually: *E. coli* ( $n = 163$  total clinical isolates in all four laboratories), *Proteus mirabilis* ( $n = 127$ ), *Klebsiella pneumoniae* ( $n = 154$ ), *Enterobacter cloacae* ( $n = 144$ ), and *P. aeruginosa* ( $n = 158$ ). Large numbers of these species were also included in the challenge set. In eight cases, individual organism-antimicrobial combinations yielded discrepancy rates much higher than the total category error rates calculated from the entire organism collection. For *E. cloacae* and the indicated agents, the rates were as follows: ampicillin-sulbactam, 5.6% very major errors; aztreonam, 4.2% very major errors; ticarcillin, 7.6% very major errors; and ticarcillin-clavulanate, 7.6% very major errors. For *P. aeruginosa*, the rates were as follows: mezlocillin, 5.1% very major errors;

TABLE 2. Results of comparisons between Vitek GNS-F6 results and broth microdilution MIC determinations with clinical isolates of *Enterobacteriaceae* and *P. aeruginosa* tested in four laboratories versus 11 antimicrobial agents

Antimicrobial	No. (%) of errors <sup>a</sup> :														
	Laboratory A (n = 231)			Laboratory B (n = 320)			Laboratory C (n = 299)			Laboratory D (n = 232)			Total (n = 1,082)		
	VM	M	mi	VM	M	mi	VM	M	mi	VM	M	mi	VM	M	mi
Ampicillin	0 (0.0)	8 (3.5)	3 (1.3)	4 (1.3)	6 (1.9)	2 (0.6)	7 (2.3)	0 (0.0)	2 (0.7)	2 (0.9)	0 (0.0)	1 (0.4)	13 (1.2)	14 (1.3)	8 (0.7)
Ampicillin-sulbactam	11 (4.8)	1 (0.4)	5 (2.2)	11 (3.4)	1 (0.3)	13 (4.1)	18 (6.0)	0 (0.0)	17 (5.7)	5 (2.2)	1 (0.4)	8 (3.4)	45 (4.1)	3 (0.3)	43 (4.0)
Aztreonam	5 (2.1)	11 (4.8)	5 (2.1)	5 (1.6)	8 (2.5)	8 (2.5)	4 (1.3)	0 (0.0)	0 (0.0)	2 (0.9)	1 (0.4)	1 (0.4)	16 (1.5)	20 (1.8)	14 (1.3)
Ciprofloxacin	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	3 (0.9)	6 (1.9)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.4)	4 (1.7)	0 (0.0)	5 (0.4)	11 (1.0)
Imipenem	0 (0.0)	9 (3.9)	3 (1.3)	0 (0.0)	9 (2.8)	3 (0.9)	0 (0.0)	4 (1.3)	2 (0.7)	1 (0.4)	6 (2.6)	0 (0.0)	1 (0.09)	28 (2.6)	8 (0.7)
Mezlocillin	2 (0.9)	7 (3.0)	7 (3.0)	7 (2.2)	1 (0.3)	11 (3.4)	4 (1.3)	1 (0.3)	9 (3.0)	6 (2.6)	0 (0.0)	12 (5.2)	19 (1.8)	9 (0.8)	39 (3.6)
Ofloxacin	0 (0.0)	2 (0.9)	1 (0.4)	0 (0.0)	12 (3.8)	7 (2.2)	0 (0.0)	1 (0.3)	1 (0.3)	0 (0.0)	4 (1.7)	0 (0.0)	0 (0.0)	19 (1.8)	9 (0.8)
Piperacillin	5 (2.2)	5 (2.2)	7 (3.0)	8 (2.5)	4 (1.3)	11 (3.4)	0 (0.0)	1 (0.3)	18 (6.0)	7 (3.0)	1 (0.4)	7 (3.0)	20 (1.8)	11 (1.0)	43 (4.0)
Ticarcillin	6 (2.6)	3 (1.3)	2 (0.9)	11 (3.4)	3 (0.9)	5 (1.6)	5 (1.7)	0 (0.0)	6 (2.0)	6 (2.6)	1 (0.4)	3 (1.3)	28 (2.6)	7 (0.6)	16 (1.5)
Ticarcillin-clavulanate	6 (2.6)	0 (0.0)	4 (1.7)	16 (5.0)	4 (1.3)	4 (1.3)	8 (2.7)	0 (0.0)	7 (2.3)	14 (6.0)	0 (0.0)	8 (3.4)	44 (4.1)	4 (0.3)	23 (2.1)
TMP/SMX	1 (0.4)	2 (0.9)	0 (0.0)	4 (1.3)	5 (1.6)	0 (0.0)	0 (0.0)	3 (1.0)	0 (0.0)	6 (2.6)	3 (1.3)	0 (0.0)	11 (1.0)	13 (1.2)	0 (0.0)
Total	36 (1.4)	48 (1.9)	38 (1.5)	66 (1.9)	56 (1.6)	70 (2.0)	46 (1.4)	11 (0.3)	62 (1.9)	49 (1.9)	18 (0.7)	44 (1.7)	197 (1.7)	133 (1.1)	214 (1.8)

<sup>a</sup> VM, very major, M, major; mi, minor.

ticarcillin, 5.7% very major errors; and ticarcillin-clavulanate, 6.9% very major errors. The rate for *P. mirabilis* with imipenem was 12.6% major errors, and that for *K. pneumoniae* with ofloxacin was 3.9% major errors. These values are based on results obtained with clinical isolates. Again, with one exception, the same general results were obtained with the common challenge set of organisms (data not shown). The exception was *K. pneumoniae* when tested against ofloxacin. As noted above, with clinical isolates, the major error rate with this combination was 3.9%; with organisms from the challenge set, the major error rate was 12.5%. We were unable to explain this difference. Initially, 140 of the 299 clinical isolates in laboratory C and 30 of 200 strains in the challenge set were inadvertently tested with the GNS-F6 card using an inoculum fourfold higher than that recommended by the manufacturer. When this error was discovered, these 170 strains were retested with a suitable inoculum density, and it is the results of this second testing that are presented in Table 2 and discussed above.

Initial testing of 140 clinical isolates with an inappropriately high inoculum in laboratory C led to significantly higher major error rates with aztreonam (16.7%), imipenem (4.0%), mezlocillin (8.4%), and piperacillin (8.4%). Disproportionately high minor error rates were observed with ampicillin-sulbactam (10%), imipenem (3.3%), mezlocillin (11.0%), piperacillin (8.4%), ticarcillin (3.3%), and ticarcillin-clavulanate (5.7%). Among the total of 125 minor errors observed in laboratory C with these six antimicrobial agents, in 104 cases (83.2%), the GNS-F6 card yielded an R or an I result, whereas the broth microdilution MIC categorized the isolate as I or S, respectively. The same patterns were noted with the 30 isolates from the common challenge set that were tested initially with an inoculum fourfold higher than that recommended by the manufacturer (data not shown).

All of the clinical isolates that had yielded very major ( $n = 197$ ) or major ( $n = 113$ ) errors with any antimicrobial agent when tested with the GNS-F6 card in the four participating laboratories (Table 2) were repeat tested with the GNS-F6 card in the coordinating study center, UMMC. In 64 cases (32.5%), among the isolates that had initially yielded 197 very major errors, a false S result was again observed on repeat GNS-F6 testing. In 130 cases (66%) among these same iso-

lates, a correct R result was obtained. Forty-four (33.1%) of the 133 major errors were corroborated upon repeat GNS-F6 testing; in 86 cases (64.7%), a correct S result was obtained. Repeat broth microdilution MIC determinations were also performed for the isolates that had yielded 197 very major errors with the GNS-F6 card as well as the isolates that yielded 133 major errors. In 189 (95.9%) and 123 (92.5%) cases, respectively, the repeat MIC was found to be the same or within  $\pm 1$  dilution increment of the initial MIC.

## DISCUSSION

The results of this investigation indicate that the currently available bioMerieux Vitek system, when used in conjunction with the GNS-F6 card according to the manufacturer's recommendations, provides accurate susceptibility test results with numerous *Enterobacteriaceae* and *P. aeruginosa* versus ampicillin, ampicillin-sulbactam, aztreonam, ciprofloxacin, imipenem, mezlocillin, ofloxacin, piperacillin, ticarcillin, ticarcillin-clavulanate, and TMP-SMX. An overall category error rate of  $<10\%$  has been established as a standard of performance for susceptibility tests (17). Included in this target percentage are very major error rates of  $\leq 1.5\%$  and major error rates of  $\leq 3.0\%$  (17). When analyzed by antimicrobial agent without respect to the organism tested, the total aggregate very major rate was found to exceed the 1.5% standard in five cases, i.e., ampicillin-sulbactam (4.1%), mezlocillin (1.8%), piperacillin (1.8%), ticarcillin (2.6%), and ticarcillin-clavulanate (4.1%). In no case did the total major error rate exceed 3.0% or was the total aggregate error rate greater than 10%. Indeed, in most instances, the error rates obtained with the study agents with the Vitek system and the GNS-F6 card were well below these limits.

Certain specific organism-antimicrobial combinations appeared to yield disproportionately high very major or major error rates. In the first category were *E. cloacae* versus ampicillin-sulbactam, aztreonam, ticarcillin, and ticarcillin-clavulanate and *P. aeruginosa* versus mezlocillin, ticarcillin, and ticarcillin-clavulanate. In the second category were *P. mirabilis* versus imipenem and *K. pneumoniae* versus ofloxacin. These observations are generally consistent with several previously published evaluations of the Vitek system (6, 9–11, 19–21, 28,



32). The higher very major error rate observed with *P. aeruginosa* and mezlocillin, ticarcillin, and ticarcillin-clavulanate may have been influenced by the fact that no intermediate MIC interpretive category has been established for these agents versus *P. aeruginosa* (18). As a result, even a 1-dilution MIC discrepancy could yield a very major error. The problem of false resistant ofloxacin results with *K. pneumoniae* with the GNS-F6 card has been previously described in the literature (5). The problem was solved by a manufacturer reformulation of the ofloxacin used in the test card (5). Updated cards containing the reformulated ofloxacin (i.e., GNS-F8) are now commercially available.

This study was intentionally conducted in clinical microbiology laboratories of four distinctly different hospital settings, an acute-care community hospital, a Veterans Administration medical center, a tertiary-care referral hospital in an academic medical center, and a pediatric hospital. It was reasoned that this diversity would provide the most rigorous challenge of the Vitek system. Surprisingly little interlaboratory variability was observed. This suggests that the utility of the Vitek susceptibility test system is not influenced by patient demographics or geographic location.

Finally, the results of this study underscore the importance of inoculum size as a determinant in the accuracy of results obtained with the Vitek system when *Enterobacteriaceae* and *P. aeruginosa* are tested. Not surprisingly, the antimicrobial agents most effected by a high inoculum were cell wall-active compounds, i.e., aztreonam, mezlocillin, piperacillin, imipenem, ampicillin-sulbactam, ticarcillin-clavulanate, and ticarcillin. Inoculum-related errors with these agents manifest as either false resistance, a major error, or minor errors in the direction of false resistance. An inoculum effect with  $\beta$ -lactam antimicrobial agents has been well documented. It is possible that two recent reports of excessive numbers of major errors when the Vitek system and the GNS-F6 card were used to test aztreonam and imipenem versus *Enterobacteriaceae* and *P. aeruginosa* were the result of an inoculum effect (1, 15). In the current study, when care was exercised to use an inoculum equivalent to that recommended by the manufacturer, accurate results were obtained with these agents.

In conclusion, the results of this multicenter clinical laboratory evaluation of the Vitek system and the GNS-F6 card indicate that this combination as currently formatted represents an accurate and acceptable means for performing susceptibility tests with the *Enterobacteriaceae* and *P. aeruginosa* versus several antimicrobial agents.

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